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TITLE: Breast Cancer Cell Line Resource Core Facility Enhancement

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The primary functions of the Breast Cancer Cell Line Resource Core Facility are to manage the breast cancer cell line repository and to assist investigators with cell culture based research. The funding received for this proposal was used for the purchase of equipment which was needed to expand and strengthen a well established and heavily utilized core facility. The LCC breast cancer cell line repository is an extremely valuable breast cancer cell line collection that is arguably one of the most extensive in existence. The repository is managed through a complete Paradox database inventory system. The individual cells are described in an Omnis 7 database which is part of a computer network with other Breast Cancer SPORE institutions. The breast cancer cell lines from the repository are made available through the core facility to investigators in other departments of Georgetown University, other U.S. institutions and foreign institutions. Over 1200 individual cell requests have been met since the opening of the core facility in November, 1989.

The purchase of two liquid nitrogen freezers and two CO_2 incubators provided critically needed cell storage and culture space to support the research of 13 LCRC breast cancer research laboratories.

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Barbara L. Zink : 7/30/95

PI - Signature Date

Ziff, Barbara L.

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INTRODUCTION

This is the final report for an infrastructure enhancement award which funded the purchase of two -150^0 C electronic cryogenic freezers with liquid nitrogen back-up, and two CO_2 incubators. This equipment provided much needed cell storage and culture space for a heavily utilized core facility.

BREAST CANCER CELL LINE RESOURCE CORE FACILITY

I. BACKGROUND

The Breast Cancer Cell Line Resource Core Facility was established as a shared tissue culture center in November, 1989, under the direction of Barbara L. Ziff, M.S. The Lombardi Cancer Center (LCC) breast cancer cell line repository is an extremely large and valuable collection of established breast cancer cell lines and genetically altered or selected clones or sublines of established breast cancer cell lines. The repository was started in October, 1988, with cells from the NCI laboratory of Dr. Marc Lippman. Dr. Lippman had been Head of the Medical Breast Cancer Section, Medicine Branch of NCI, NIH, prior to his appointment as Director of the Lombardi Cancer Center. The LCC breast cancer cell line repository is now managed as a central resource by the LCC Breast Cancer Cell Line Resource Core Facility. The core facility was funded as one of the four shared resources of the Specialized Program of Research Excellence in Breast Cancer (SPORE) which was awarded to the LCC in 1992. In 1993, the core facility received additional funding as a new shared resource in the renewal of the LCC Cancer Center Support Grant (CCSG). In 1995 the core facility was again funded as a shared resource of the SPORE in Breast Cancer grant in a competitive resubmission. The core facility has developed steadily over the past six years. It has been extensively used by members of all of the programs of the CCSG, including the Breast Cancer Program, the Invasion and Metastasis Program and the Growth Regulation of Cancer Program, and by each of the programs in the Breast Cancer SPORE.

II. PURPOSE

The goals of the Breast Cancer Cell Line Resource Core Facility are to manage the LCC breast cancer cell line repository, to provide a carefully maintained, monitored, and well equipped tissue culture space to breast cancer investigators, and to offer a number of tissue culture related services. The cell line repository described in this report is quite large. It contains cells derived from many types of cancers, and notably includes a breast cancer cell line collection that is arguably one of the most extensive in existence. It is an additional goal of the core facility to make the breast cancer cell line repository available to investigators in other cancer research centers.

A. Description of Services

A1. Breast Cancer Cell Line Repository

The management of the breast cancer cell line repository is one of the main responsibilities of the Breast Cancer Cell Line Resource facility. Facility personnel maintain both growing and frozen cell stocks. The frozen stocks are replenished as required through the expansion and freezing of cells and subsequent testing for viability. As new breast cancer cell lines or clones are developed or acquired by investigators, they are added to the breast cancer cell line repository stock. The core facility maintains a registry of LCC cell names in order to coordinate the sequential naming of cells developed in the LCC.

The large volume of the cell repository and the rapid pace of on-going research present a considerable challenge in freezer space management and record keeping. Prior to the support received from the Army, the three liquid nitrogen freezers maintained as shared space by the core facility, with a combined capacity of 48,000 frozen vials of cells, were shared by 17 laboratories and were essentially full. The additional two freezers purchased through this award have greatly alleviated the over-crowding of the repository and have allowed new LCC researchers to acquire space. The laboratories of 30 principal investigators now share the five freezers of the core facility.

Because the repository is used by a large number of researchers, a centralized inventory system is essential for efficient management. A comprehensive inventory of the repository was undertaken in 1991 and is now completed and continuously updated. The cell inventory database was created by the LCC Biostatistics Core Facility in 1991 to specifically meet the needs of the repository and is still in use. The initial inventory efforts focused on storing basic identification, location and quantity information on each cell line. As researchers add or remove repository cells this information is recorded on forms kept on top of the freezers. Data from these forms is used to keep the inventory database up to date.

A second database, the Resource Database, contains information about the source of the cells (patient data such as race, age, diagnosis, site of tumor), hormone receptor and response status, growth properties and tumorigenicity, genetic alteration, resistance phenotype, growth factor and growth factor receptor expression and response, and oncogene expression. Also included in this database are records of test results, including mycoplasma assays, and helper virus rescue assays. This database can be searched for any of the included variables, in order to pull out information on particular cells or to select cells with a defined set of characteristics. Much of the information is being entered in a numerical code which will allow statistical retrieval and analysis of the data to be performed.

The Resource Database is linked to a report form for each cell line which includes all of the

available data except test results. The hard copy of this report form, or a document consisting of any or all of the report forms, will be made available upon request to all researchers in the LCC and to other cancer research facilities. It is anticipated that this information will facilitate collaborations between LCC Breast Cancer investigators and breast cancer investigators in other institutions.

Requirement for Additional Liquid Nitrogen Freezer Storage Space

The equipment purchased with this award is housed in the Breast Cancer Cell Line Resource Core Facility. The equipment provides vitally needed space for the culture and cryostorage of cells used in the breast cancer research the LCC. A survey of liquid nitrogen freezer space needs was conducted among the breast cancer researchers of the Lombardi Cancer Center in October, 1993. All of these researchers responded with a request for more space. The need for more incubator space was not specifically addressed in this survey. However, it was clear that the amount of cell culture based research planned by these investigators would require additional incubator space. The principal investigators, titles, funding agencies and project descriptions of a few of the breast cancer research projects which required this additional space are described below:

P.I.: Ruth Lupu, PhD., Assistant Professor, Departments of Biochemistry and Molecular Biology

Project Title/Funding Agency: Involvement of the erbB-2 Ligand in Breast Cancer Tumor Progression/NIH

In the last decade we have come to understand that the growth of cancer cells in general and of breast cancer in particular depends, in many cases, upon small proteins termed growth factors that will bind and then activate their growth factor receptors. One of these growth factor receptors is the *erbB*-2 receptor which plays an important role in the prognosis of breast cancer and is expressed at very high levels in nearly 30% of human breast cancer patients. While evidence accumulates to support the relationship between *erbB*-2 over expression and poor overall survival in human breast cancer, understanding of the biological consequence(s) of *erbB*-2 over expression remains elusive. In addition, the receptor is necessary for the maintenance of the malignant phenotype of cells transformed by *erbB*-2.

Dr. Lupu's discovery of an *erbB-2* ligand (*gp30*) has allowed her to identify a number of related but distinct biological endpoints which appear responsive to signal transduction through the *erbB*-2 receptor. These endpoints of growth, invasiveness, and differentiation have clear implications for the emergence, maintenance and/or control of malignancy, and represent established endpoints in the assessment of malignant progression in human breast cancer. Preliminary studies *in vitro* have shown that *gp30* induces a biphasic growth effect on cells with

erbB-2 over-expression. Strikingly, Dr. Lupu has recently observed that the *erbB*-2 signaling pathway can be modulated by estrogen acting through the estrogen receptor (ER). Conversely, she observed that down regulation of *erbB*-2 by estrogen can be blocked by *gp30* acting through the *erbB*-2 receptor. Clearly, mechanistic aspects of the *erbB*-2/ligand interaction need to be understood from a therapeutic standpoint, and may furthermore provide additional insights into treatment synergy for certain patients, or enhance treatment regimens for a large number of women. Understanding the mechanism(s) through which these interactions occur will provide a rational framework with which to examine the therapeutic relevance of the interaction, and may serve to expose other therapeutic targets. Dr. Lupu's laboratory has introduced the ligand cDNA into a variety of normal and cancer breast epithelial cells. Their research studies are designed to define the relevance of *gp30* in breast cancer tumor progression. They estimated that they would need one additional tower of ten boxes to store the cell lines generated in this project (1000 vials).

P.I.: James Zwiebel, M.D., Assistant Professor of Medicine, Department of Hematology

Project Titles/Funding Agencies:
Implants of Genetically Modified Endothelial Cells/NIH
Prototype Gene Therapy Deliver System/NIH
Human Pulmonary Endothelium Gene Transfer and Response/NIH

Dr. Zwiebel's laboratory is engaged in the continual development of retroviral packaging cell lines for the purpose of introducing new genes into cells. They subsequently transduce various types of cells with the retroviral vectors. Due to the fall off of vector titer with passage in culture, master seed lots of each packaging cell line at low passage must be maintained in liquid nitrogen. All of these cell lines are extremely valuable reagents, both for Dr. Zwiebel's laboratory and for other investigators. This laboratory therefore has a steady, growing requirement for additional liquid nitrogen space. Dr. Zwiebel expressed an immediate need for two additional storage towers of ten boxes each (2000 vials).

Erik Thompson, Ph.D., Assistant Professor, Department of Anatomy and Cell Biology.

Project Title/Funding Agency: Characterization of 72 kDa Type IV Collagenase Activator Associated With Human Breast Cancer Invasiveness/NIH

Dr. Thompson's laboratory is dedicated to the molecular and cellular analysis of metastatic progression in human breast cancer. Using established human breast cancer cell lines, they have characterized a model system which represents the progression from hormone-dependent, relatively benign human breast cancer to the more invasive and metastatic hormone-independent form. Molecular analyses of this system, in conjunction with analysis of human breast tumor specimens, has implicated certain extracellular protease systems in the metastatic process.

The approach of the laboratory is to analyze certain protease system components, establish relationships and hypotheses, and test these in our model system using inhibitor analysis in conjunction with transfection of either sense or antisense constructs. Transfection analyses require the use of multiple cell lines. For each transfection it is necessary to generate a large number of individual clones carrying either the control vector or the expression construct. In addition to these cell lines, they are continuously attempting to isolate and characterize cellular populations from freshly biopsied human breast tumors. Dr. Thompson's cell freezer requirements are accordingly substantial. He estimated that he would need an additional 5 freezer boxes each year for the next three years (1500 vials).

Kevin Cullen, M.D., Assistant Professor of Medicine, Department of Medical Oncology.

Project Title/Funding Agency: Stromal Growth Factor Expression in Breast Cancer/NCI

Dr. Cullen's laboratory is engaged in ongoing studies on the expression and regulation of peptide growth factor genes in breast cancer stroma. Specifically, they have examined the expression of mRNA species for Insulin-like growth factors, which have important biologic functions in models of breast tumor growth. The fundamental hypothesis of their work is that peptide growth factors produced by breast cancer stroma are instrumental in the overall regulation of breast cancer growth. Dr. Cullen's preliminary data indicate that stromal fibroblasts from normal breast and benign lesions express Insulin-like growth factor I (IGF-I), the form of the growth factor which has important physiologic growth promoting functions in adolescents and normal adults. In contrast, fibroblasts from breast cancers express IGF-II, which is felt to be a fetal growth factor with no known function in the adult. Further, they have shown in co-culture experiments that fibroblasts derived from normal breast can be converted to the phenotype seen in breast cancers when grown in the presence of breast cancer epithelium.

Since Dr. Cullen observes selective expression of IGF-I in benign breast stroma while IGF-II is a product of tumor stroma, he is currently investigating the biological function of both IGF-I and IGF-II in breast cancer through a series of transfection studies. Dr. Cullen's laboratory has made several IGF-II mutants which contain alterations in binding domains for either the IGF-I or IGF-II receptor. With these mutants, they hope to be able to better determine the differential effects of IGF-I and IGF-II in promoting tumor growth. Dr. Cullen also hopes to be better able to determine whether the IGF-I receptor or the IGF-II receptor mediates the signals responsible for enhanced tumor growth.

Because of his work with multiple fibroblast lines, as well as the numerous IGF mutant cell lines, Dr. Cullen has an increasing need for liquid nitrogen freezer space. Presently, he has had difficulty obtaining adequate storage space for the lines his laboratory is generating, and the additional long term cell storage capacity provided by this infrastructure grant would greatly facilitate his studies. Dr. Cullen requested an additional 10 boxes of storage space (1000 vials).

Mathew Ellis, M.D., Ph.D., Postdoctoral Fellow, Laboratory of Dr. Kevin Cullen, Lombardi Cancer Research Center.

Dr. Ellis' principal research aim is to understand the role of insulin-like growth factors (IGF) in human malignancy. A key aspect of this research involves transfection studies using retrovirus mediated gene transfer. He now has a number of packaging cell lines containing different IGF constructs, as well as stocks of virus supernatants. Additionally he has a number of breast cancer cell lines transfected with these IGF retroviruses. Liquid nitrogen space is essential for this work so that all these reagents can be stored. As he is expanding the number of IGF mutants that he is working on, he faces an acute shortage of liquid nitrogen space in the near future. Dr. Ellis requested an additional five boxes of storage space (500 vials).

Anton Wellstein, M.D., Associate Professor, Department of Medicine, Department of Pharmacology

Project Title/Funding Agency: Inhibition of Heparin Binding Growth Factors/NIH

Dr. Wellstein is pursuing studies with genes that are expressed during the malignant progression of breast cancer. His laboratory has generated and will be generating a large number of different, engineered, cell lines. To date, they have generated over 40 clonal human breast cancer cell lines (MCF-7 clones) from transfections with a growth factor gene (pleiotrophin) that is expressed in human breast cancer samples. The long-term storage required for these existing cell lines and the dozens of other clonal lines that Dr. Wellstein's laboratory is in the course of generating requires that they expand their current liquid nitrogen freezing capacity. One box of 10x10 vials is sufficient to hold a maximum of 8 cell lines with 12 aliquots of one freeze-down. One transfection normally generates 30 clonal cell lines (15 clonal cell lines from a transfection with the empty vector as a control and 15 clonal cell lines from a transfection with the vector containing the gene of interest). Due to the fact that they use approximately 10 standard human breast cancer cell lines as recipients of engineered genes and plan to do work on 5 different genes, they requested approximately one half of a liquid nitrogen freezer for this particular project (approximately 10,000 vials).

Robert Dickson, Ph.D., Associate Professor, Department of Anatomy and Cell Biology.

Project Titles/Funding Agencies: MDGF1 and Its Receptor In Human Breast Cancer/American Cancer Society
Regulation of Invasion/NCI
TGF-Alpha and Its Receptors in Breast Cancer/American Cancer Society

Dr. Dickson's laboratory is establishing cell lines from transgenic mouse mammary tumors.

They are also performing FGF transfections of T47D cells and LacZ transfections into various breast cancer cell lines. Other genes which they are transfecting into breast cancer cell lines include: macrophage chemoattractant, myc, mutant steroid receptors. Dr. Dickson estimated that his laboratory would require seven additional boxes of space for cryostorage during the next year (700 vials).

Michael D. Johnson, Ph.D., Postdoctoral Fellow, Laboratory of Dr. Robert Dickson, Lombardi Cancer Research Center

Project Title/Funding Agency: The Mechanisms Underlying Resistance To Pure Antiestrogens in Breast Cancer/Susan G. Komen Breast Cancer Research Foundation

Dr. Johnson is engaged in an investigation of the mechanisms involved in the acquisition of resistance to antihormonal therapy and the development of the hormone independent phenotype in human breast cancer. This work involves the generation of breast cancer cell sub-lines that exhibit these properties, and their subsequent characterization to determine the changes in the cells that have allowed this behavior. Each new subline that is generated is unique and must therefore be cryopreserved in a liquid nitrogen freezer. A number of such sublines must be analyzed to allow any change that is found to be linked with confidence to the resistant behavior. Therefore, even with the maintenance of minimal stocks of each variant a considerable amount of freezer space is required. His previous allocation of freezer space was nearing capacity, in spite of a rigorous policy of discarding vials of cells no longer of interest. If more freezer space had not become available Dr. Johnson would have found it increasingly difficult to conduct his research. He requested an additional 3 boxes of storage space (300 vials).

Yuenian Eric Shi, Ph.D., Postdoctoral Fellow, Laboratory of Dr. Robert Dickson, Lombardi Cancer Research Center

Project Title/Funding Agency: A Novel Matrix Metalloproteinase in Hormone-Dependent Breast Cancer/NIH

Metalloproteinases are extracellular matrix-degrading enzymes that have been implicated in the early stages of breast cancer metastases. Both 72 kDa and 92 kDa species of metalloproteinases have been described in breast cancer. Dr. Shi has isolated and described an apparently new metalloproteinase from breast cancer cell lines. This enzyme is secreted as a complex of approximately 80 kDa in size. As part of his characterization of this molecule, Dr. Shi is generating monoclonal antibodies against it. These antibodies will be used to detect the enzyme in breast tumor biopsies and to compare its expression in tumors to that of other proteases. Dr. Shi requested 7 additional boxes of liquid nitrogen freezer space to store the hybridoma cells and subclones generated in the search for monoclonal antibodies against this metalloproteinase (700 vials). (Dr. Shi has now moved to another university, but this work is continuing in Dr.

Dickson's laboratory.)

Fran Kern, Ph.D., Assistant Professor, Department of Biochemistry

Project Title/Funding Agency: Genetic Mechanisms of Breast Tumor Progression/NCI

Dr. Kern's laboratory is currently involved in a number of studies involving transfection of growth factor genes into breast carcinoma cell lines. They are also screening metastatic, estrogen-independent, and Tamoxifen-resistant cell lines to identify genes which are responsible for those phenotypes. They estimated that they would have need for liquid nitrogen storage space of 5 boxes for the clonal cell lines which would be produced by this research over the next two years (500 vials).

All of these investigators have now acquired additional cell storage space in the new core facility cryogenic freezers which were purchased with this award.

A2. Shared Tissue Culture Areas

The new Georgetown University Research Building, which was fully opened in April, 1995, houses most of the researchers of the LCC. The core facility provides and maintains shared tissue culture rooms on three floors of this building. The core facility also provides and maintains a quarantine facility which is used for the isolated culture of newly introduced cells until they can be tested for mycoplasma contamination. The hoods and incubators of the shared areas have been heavily used since the move of the core facility into the new building, and this level of usage will certainly continue. The two CO_2 incubators which were purchased with funding from this proposal are now used in these shared rooms. Several of the individual CO_2 incubators in these shared rooms are dedicated to special usage, such as transfections, temperature specific growth of temperature sensitive mutant lines, CO_2 free growth of primary cell lines, or radioactive work.

A3. Cell Requests

The LCC breast cancer cell line repository provides cells upon request to individual investigators in the LCC and to outside investigators in the United States and abroad. Cells are provided as either growing or frozen cultures. There has been a significant demand for this service, as shown in Table 1.

Table 1. Cell Line Requests and Recipients, 8/89 to 12/94

DATES	# CELL REQUESTS	RECIPIENTS: LCC	U.S.	FOREIGN
8/89 - 12/89	140	24	4	2
1/90 - 12/90	245	34	25	9
1/91 - 12/91	163	28	18	7
1/92 - 12/92	138	34	30	7
1/93 - 12/93	232	25	18	9
1/94 - 12/94	265	27	2	2
TOTAL	1183			

A4. Mycoplasma Testing

Mycoplasma testing has been provided as a service to LCC investigators since April, 1990. Because so much of the research of the LCC is based upon tissue culture, the detection of mycoplasma infection is considered a quality control measure of the utmost importance. The assay performed uses a Gen-Probe detection kit which is based upon nucleic acid hybridization of a ^{3}H DNA probe complementary to the ribosomal RNA of *Mycoplasma* and *Acholeplasma* RNA. This probe detects all species which commonly infect laboratory cultures. Results can be obtained within three hours. Mycoplasma tests are performed on all cells arriving in the LCC from outside laboratories and as a routine check on existing LCC stock. The demand for this service has been very high, as shown in Table 2.

Table 2. Mycoplasma tests performed, 4/90 through 6/95

DATES	# CELL LINES	# LABORATORIES REPRESENTED	AVG. # TESTS/MO.
4/90 - 6/90	24	6	8.0
7/90 - 6/91	114	11	9.5
7/91 - 6/92	260	20	22.6
7/92 - 6/93	170	19	14.1
7/93 - 6/94	544	32	45.3
7/94 - 6/95	263	24	21.9

A5. Helper Virus Rescue Assay

There is an increased utilization by LCC personnel of retroviral technology to achieve efficient gene transfer. A clear set of guidelines has been established on the research floor of the LCC which must be followed by all persons performing procedures which require Biosafety Level 2 containment. One of these regulations is that cell lines of questionable biohazardous potential must be certified helper virus free before being moved from Biosafety Level 2 containment to Biosafety Level 1. The helper virus rescue assay required under these circumstances has been offered by the core facility since August, 1991. This assay is based upon the ability of helper viruses to rescue the BAG vector from NIH 3T3 cells. Test supernatant is incubated with BAG 3T3 cells. If helper virus is present the BAG vector will be packaged in viral particles containing the envelope of the helper virus. The rescued BAG vector is tested for its ability to confer lacZ expression or G418 resistance upon normal NIH 3T3 cells. The assay takes two weeks to complete. The first assay performed detected a cell line that was both helper virus positive and transforming. This assay clearly provides a much needed safeguard for research involving retroviral technology. The demand for this service has been significant.

A6. DNA Fingerprinting of Breast Cancer Cell Lines

The development of a simple and inexpensive method of DNA fingerprinting has provided an important quality control measure for the cell lines of the repository. Because most laboratories are using multiple cell lines, there is always the concern of potential cell line cross-contamination. The core facility has arranged for both karyotyping and isozyme typing on various cell lines, by the Children's Hospital of Michigan, as it has been deemed necessary or desirable. However, these procedures, particularly karyotyping, are

expensive, and results can take up to six months to receive.

Highly polymorphic genetic loci known as repetitive minisatellite regions, or as variable number tandem repeat (VNTR) polymorphisms, show multiallelic variation in the number of repeat units and correspondingly high heterozygosity. PCR amplification of these DNA regions can be used to "fingerprint" cell lines. The core facility has prepared DNA from our commonly used cell lines and has determined the appropriate probes to produce distinctive patterns for each cell line. The probes selected are for the Apolipoprotein B-100 (Apo B-100, the major protein constituent of low-density lipoproteins), and Locus D1s80. The Apo B-100 locus has been shown to have a heterozygosity of 78% and D1s80 has been shown to have a heterozygosity of 80.8%. This probe has produced a distinct pattern for each breast cancer cell line tested to date. While not a conclusive means of identification, the distinctive patterns of two cell lines can be used to rule out cross-contamination between those lines.

This technique was developed primarily as a quality control measure for the cells of the repository, but it is also now made available to researchers as a service of the core facility.

A7. Cell Freezing Service

This service of the core facility was introduced in May, 1994. Core facility personnel have in the past been responsible for the expansion and freezing of cell stocks designated as belonging to the core facility, but not those of individual investigators. Optimal management of the breast cancer cell repository is facilitated by as much centralization as possible of the freezing of cells, avoiding the duplication of stocks and efforts. This service has proved to be an extremely useful addition to the core facility.

A8. Cell Culture Service

This service of the core facility was also introduced in May, 1994. Researchers requesting cells from the core facility frequently ask to receive one or more growing flasks rather than a frozen vial. The core facility has also received requests to take over the culture of an investigator's cells while they are away, or to provide the number of flasks of cells required for a particular experiment. While most requests are for a few flasks, the core facility is also available to assist researchers with bulk cell production. This service has been regularly used by many of the laboratories in the LCC, including eight breast cancer research laboratories.

B. Operation

B1. Administrative Control

The Breast Cancer Cell Line Resource Core Facility is a core facility of

the Lombardi Cancer Research Center. The director of this facility reports directly to Dr. Marc Lippman, Lombardi Cancer Research Center Director, concerning the administration of the facility.

B2. Policies and Priorities

It is the policy of the Breast Cancer Cell Line Resource Core Facility to make its resources and services available to all members of the Lombardi Cancer Research Center. When it is necessary to set priorities due to the limitations of space or personnel, priority is given to peer-reviewed funded LCC members. The cells of the core facility cell repository are also made available to investigators throughout the U.S. and abroad.

B3. Personnel

B3a. Core facility Director

Barbara L. Ziff, M.S., Research Associate, Department of Anatomy and Cell Biology, is the director of the core facility and is primarily responsible for the operation of the facility. Ms. Ziff was instrumental in the development of the Breast Cancer Cell Line Resource Core Facility, and has directed it and continued to develop it since its opening in November, 1989. She has extensive experience in a wide range of tissue culture techniques, monoclonal antibody production, protein purification and characterization, cDNA cloning and sequence analysis, polymerase chain reaction techniques and other molecular biology research methods.

B3b. Technical Support

Min Lee, M.S., Research Assistant III, Lombardi Cancer Research Center, joined the staff of the Breast Cancer Cell Line Resource Core Facility on October 1, 1993. Prior to this, Ms. Lee was employed as Biologist II for three years at the American Type Culture Collection in Rockville, Md. She is very experienced in tissue culture techniques, as well as in molecular biology techniques. She has excellent laboratory skills and has been actively involved in the continued development and implementation of new tissue culture related services.

Evalina Garkovenko, M.D., Research Assistant II, Lombardi Cancer Research Center, has been employed by the Breast Cancer Cell Line Core Facility since May, 1995. Dr. Garkovenko completed a two year post doctoral fellowship at Georgetown prior to beginning work in the core facility. She has excellent tissue culture and laboratory skills and is becoming very skilled in the performance of core facility services.

B4. Cost Effectiveness

The Breast Cancer Cell Line Resource Core Facility is extremely cost effective for LCC investigators. With so much of the day to day tissue culture work performed by a core facility, a great deal of duplication of effort is eliminated. Each investigator is relieved of the necessity of maintaining his or her own stocks of commonly used cell lines and may, if they wish to do so, rely completely upon the centralized inventory databank for their cell storage records. At present, facility personnel are performing all of the time consuming tasks of the different services of the facility for a large number of laboratories. This avoids the necessity for each laboratory to develop expertise in the various assays provided. Additionally, the equipment of the facility is in almost constant use. This is very efficient in that fewer pieces of equipment are serving more people.

B5. Quality Control

Quality control in matters of cell culture is of the highest importance and is therefore a major emphasis of the facility. The growth curve analysis, mycoplasma tests, helper virus rescue assays, DNA fingerprinting, karyotyping, isoenzyme analysis and MAP testing discussed above are all very important quality control measures. The assays offered by the facility are carefully supervised and performed by highly trained and experienced personnel. Very strict rules for the handling of cells in culture are enforced. Only one cell line at a time is brought into a tissue culture hood by core facility technicians, and separate media bottles are maintained for each cell line. The shared tissue culture space and equipment are well maintained and closely monitored. All personnel using the facility are required to be adequately trained, and all incoming cells must first be placed into quarantine until they are determined to be mycoplasma free.

B6. User Fees

The core facility recovers approximately 58% of it total costs from user fees. User fees for facility services and shared tissue culture space usage are based on the cost of supplies and labor. The charges for individual tissue culture services are listed below:

Mycoplasma Tests: $35.00 per cell line.
Helper Virus Rescue Assay: $25.00 per cell line.
Cell Requests: No charge for Georgetown University investigators. Outside investigators are asked to pay shipping costs.
Cell Repository: No charge.
Cell Freezing: $25.00 per cell line.
Cell Culture: $10.00 per T75 flask.

DNA Fingerprinting: $39.15 for the first gel, with 7 PCR samples
$9.85 for an additional gel, with 9 PCR samples

IV. SCOPE AND PURPOSE OF PROPOSED SERVICES AND FUTURE DIRECTIONS

A. New Services

The number of services provided by the Breast Cancer Cell Line Resource Core Facility has recently been expanded by the addition of the Cell Freezing service, the DNA Fingerprinting service, and the Cell Culture service. These services are logical extensions of the work previously performed in the facility, and have furthered the goal of making this facility increasingly useful to LCC breast cancer researchers.

B. Discontinued Services

The genetic labeling of breast cancer cells with lacZ by transfection with a lacZ containing plasmid vector, and the production of conditioned media, had been proposed in the past as developing services of the core facility. However, the demand for these services did not develop as anticipated and there are no current plans to further develop these services.

C. Developing Service

The establishment of primary breast cancer cell lines is a developing service of the core facility. The core facility has begun to collaborate with LCC breast cancer researchers who wish to have primary breast cancer cell lines established from tumor samples. The Tumor Bank Core Facility, which is also funded as a core facility of the Breast Cancer SPORE grant, is assisting us in obtaining tissue samples. Initially, the core is accepting samples of breast primary and metastatic tumors, and of normal breast tissue adjacent to the breast tumors, with the goal of developing expertise in the establishment of cell lines from these samples. Ultimately, the core will work on a collaborative basis with breast cancer researchers and endeavor to develop only cell lines that are of specific value to a particular research project.

V. INTERACTIONS WITH PROGRAMS AND OTHER CORE FACILITIES

The Breast Cancer Cell Line Resource Core Facility is a central and important asset to the researchers of the LCC, and must meet the current tissue culture related needs of the researchers. With this goal, every effort is made to foster communication and interactions between the personnel of the core facility and other LCC program and core facility leaders and personnel.

The core facility is always open to new ideas for services that meet the needs of the researchers.

Questionnaires have been periodically circulated to elicit ideas and suggestions from core facility users on continuing services and the development of new services. In addition, the director of the core facility attends the regular monthly meeting of Lombardi Cancer Center senior staff, and presents a report each month on the status of the core facility. This meeting has proved to be an excellent forum for the development of new ideas and the generation of useful feedback.

A. Program and Core Facility Usage

The services and/or facilities of the Breast Cancer Cell Line Resource Core Facility are used by members of all of the nine programs in the LCC Cancer Center Support Grant and by members of all of the five programs in the Breast Cancer SPORE grant. Members of five of the nine other LCC core facilities either directly use or interact with the Breast Cancer Cell Line Resource Core facility. LCC administrative staff provide support to the Breast Cancer Cell Line Resource Facility in the form of budget, purchasing, personnel and administrative management. The core facility therefore has a very balanced and broadly based pattern of usage by and interaction with members of the cancer center.

B. Effect of the Breast Cancer Cell Line Resource Core Facility on the Stimulation of Scientific Interaction

The breast cancer cell line resource database which is being developed by the Breast Cancer Cell Line Resource Core Facility is proving to be an extremely useful tool for the stimulation of scientific interaction between LCC research programs and between these programs and other Georgetown University researchers. The database provides information to researchers that has previously not been accumulated into one easily accessible collection of information. With the current establishment of a computer network between the LCC Breast Cancer SPORE and the three other Breast Cancer SPORE institutions, this database will be enormously useful in fostering scientific interactions.

C. Scientific and Oversight Committee

The overall operation of the Breast Cancer Cell Line Resource Core Facility is facilitated through a Scientific and Oversight Committee. This committee meets approximately four times per year and decides matters of policy on issues such as the prioritization of usage of the facility, establishment of fees and the development of future requirements of the facility. The members of the committee are listed below:

Barbara L. Ziff, M.S.: Director, Breast Cancer Cell Line Resource Core Facility, Research Associate, Department of Anatomy and Cell Biology

Robert Clarke, PhD: Director, LCC Animal Core Facility, Assistant Professor, Department of Physiology and Biophysics

Robert B. Dickson, PhD: Associate Professor, Dept. of Anatomy and Cell Biology

Karen K. Huff, M.S.: Research Administrator, Lombardi Cancer Center, Research Associate, Department of Microbiology

Carolyn K. Hurley, PhD: Associate Professor, Department of Microbiology

Usha Kasid, PhD: Associate Professor, Department of Radiation Medicine

James A. Zwiebel, MD: Assistant Professor of Medicine, Department of Hematology

CONCLUSIONS

The Breast Cancer Cell Line Resource Core Facility is an extremely large and well utilized shared resource of the LCC. The core facility has continued to grow and develop during the last year. The core has moved into the newly completed Research Building on the Georgetown University campus, and is providing expanded shared tissue culture space to researchers in the new building. Three new services, cell culture, cell freezing and DNA fingerprinting of cell lines, have been added to the core facility services during the last year. The establishment of primary cell lines from breast tumors is a developing service.

The funds obtained through the Army Grant Infrastructure Enhancement award have been spent as authorized to obtain two -150 ^{0}C electric cryogenic freezers with liquid nitrogen back-up, and two dual chamber CO_2 incubators. This equipment has provided much needed additional cell storage space and cell culture space in the core facility. The additional space has been made available to LCC breast cancer researchers and is being fully utilized at this time.

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